

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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Application No: 10/078,278  
Confirmation No: 3427  
Applicant: Robert E. Wagner, Jr.  
Filing Date: February 20, 2002  
Group Art Unit: 1634  
Examiner: Sarae L. Bausch  
Attorney Docket No: 007274-01  
Customer No: 36,234

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

**DECLARATION  
37 C.F.R. 1.132**

Dear Sir;

This Declaration is made by Dr. Robert E. Wagner, Jr., inventor of the present application. This Declaration is submitted in conjunction with an amendment and response to the office action having a mailing date of June 1, 2007.

I, Robert E. Wagner, state and declare as follows:

1. I am an employee of Gene Check Inc. and the inventor of the present invention.

2. As inventor of the present invention, I am highly familiar with the art pertaining to MutS proteins.

3. At the time the present application was filed, it was understood by one of skill in the art that the ability of MutS proteins to bind nucleotide mismatches is complex. Not all nucleotide duplex structures are recognized by MutS.

4. In support of the position asserted in Paragraph 3, the following statement is provided:

In 1995, two series of experiments were initiated in an attempt to demonstrate the ability of *E. coli* MutS to recognize single base pair mismatches in RNA/DNA hybrid duplexes. A series of *in vivo* experiments was performed in the laboratory of Miroslav Radman, Ph.D., co-inventor of the present application, in Paris, France. Concurrently, a set of *in vitro* experiments was performed in my laboratory in Gene Check, Inc., Fort Collins, CO. Our experiments utilized synthetically manufactured oligonucleotides annealed to form duplexes of four different types:

- i. DNA/DNA duplexes without mismatches, i.e., perfectly paired
- ii. DNA/DNA duplexes containing a centrally located G:T mismatch (the G:T is the mismatch best recognized by *E. coli* MutS)
- iii. RNA/DNA duplexes without mismatches
- iv. RNA/DNA duplexes with a centrally located G:T mismatch

All oligonucleotides were, except for the presence or absence of the mismatch, of identical sequence. One strand of each duplex contained a 5'-biotin adduct for DNA detection as described in Wagner et al. (1995, Nuc Acids Res 23:3944-3948). Although normal binding of G:T mismatch containing DNA/DNA duplexes was observed, no binding of mismatch containing RNA/DNA duplexes could be detected.

The results of the *in vivo* experiments confirmed those of the *in vitro* experiments.

Conclusion: *E. coli* MutS does not recognize mismatches in RNA/DNA duplexes.

5. At the time the present application was filed, it was understood by one of skill in the art that RecA bound to a D-loop of a three stranded DNA structure inhibited enzymatic action on that DNA structure.

6. In support of the position asserted in Paragraph 5, the following exhibit(s) are attached:

A. Ferrin et al., (1991, Science 254:1494-1497) found that RecA mediated triplex formation inhibited DNA polymerase filling of overhanging 5' ends. The abstract to the paper states, "These three-stranded complexes were protected from the action of DNA polymerase." In fact, in order to allow the fragments to be substrates for DNA ligase, Ferrin et al. first extracted the RecA with phenol/chloroform.

B. In a later work, (Ferrin et al., 1998, PNAS 95:2152-2157) it was shown that triple stranded RecA mediated complex formation also inhibited Eco RI methylase action. From that abstract: "The technique is based on the ability of RecA protein from *Escherichia coli* to pair an oligonucleotide to its homologous sequence in duplex DNA to form a three-stranded complex. This complex is protected from Eco RI methylase . . ."

C. The results of work in our laboratory using circular plasmid DNA and RecA filaments formed using oligonucleotides complementary to a region of the plasmid

DN containing a restriction endonuclease cutting site demonstrated that triplex structures, in the presence of RecA and formed by RecA mediated homology searching, inhibited restriction endonuclease cleavage of the cutting site included in the three stranded complex.

7. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing thereon.

Inventor's Signature REW-2 Dated: October 25, 2007

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